

FIG. 1

sequence\_AA\_2D12.5\_variable domains.txt

```
>2D12.5VL_MOUSE
(1) QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGNNRPPGVPARFSGSLIGDKAALTIAGTQTED
    EAIYFCALWYSNHWFVGGGTRLTVLG

(2) CDR1 - RSSTGAVTTSNYAN
(3) CDR2 - GNNNRPP
(4) CDR3 - ALWYSNHWF

>2D12.5VH_MOUSE
(5) QVKLQESGPGVLVQPSQSL SITCTVSGFSLTDYGVHWVRQSPGKGLEWLGVIWSGGGTAYTAAFISRLNIY
    KDNSKNQVFFEMNSLQANDTAMYYCARRGSYPYNYFDVWGQGTTVTVSS

(6) CDR1 - DYGVH
(7) CDR2 - VIWSGGGTAYTAAFIS
(8) CDR3 - RGSYPYNYFDV
```

FIG. 2

## Translation of 2D12.5 VH variable genes

			10	20	30	40	50	
(9)	2d12.5 VH native hybridoma	1	..... ..... ..... ..... ..... ..... ..... .....					
(10)	2d12.5 VH native cloned	1	..... ..... ..... ..... ..... ..... ..... .....					50
(11)	2d12.5 VH N87D_cloned	1	..... ..... ..... ..... ..... ..... ..... .....					50
(12)	2d12.5 VH N87D_G53C_cloned	1	..... ..... ..... ..... ..... ..... ..... .....					50
(13)	2d12.5 VH N87D_G54C_cloned	1	..... ..... ..... ..... ..... ..... ..... .....					50
(14)	2d12.5 VH N87D_G55C_cloned	1	..... ..... ..... ..... ..... ..... ..... .....					50
			60	70	80	90	100	
	2d12.5 VH native hybridoma	51	WSGGGTAYTAAAFISRLNIYKDNSKNQVFFEMNSLQANDTAMYYCARRGSY					100
	2d12.5 VH native cloned	51	..... ..... ..... ..... ..... ..... ..... .....					100
	2d12.5 VH N87D_cloned	51	..... ..... ..... ..... ..... ..... ..... .....			D		100
	2d12.5 VH N87D_G53C_cloned	51	..... ..... ..... ..... ..... ..... ..... .....			D		100
	2d12.5 VH N87D_G54C_cloned	51	..... ..... ..... ..... ..... ..... ..... .....			D		100
	2d12.5 VH N87D_G55C_cloned	51	..... ..... ..... ..... ..... ..... ..... .....			D		100
			110					
	2d12.5 VH native hybridoma	101	PYNYFDVWGQGTTTVTVSS					118
	2d12.5 VH native cloned	101	..... ..... ..... ..... ..... ..... ..... .....			A		118
	2d12.5 VH N87D_cloned	101	..... ..... ..... ..... ..... ..... ..... .....			A		118
	2d12.5 VH N87D_G53C_cloned	101	..... ..... ..... ..... ..... ..... ..... .....			A		118
	2d12.5 VH N87D_G54C_cloned	101	..... ..... ..... ..... ..... ..... ..... .....			A		118
	2d12.5 VH N87D_G55C_cloned	101	..... ..... ..... ..... ..... ..... ..... .....			A		118

FIG. 3A

## 2D12.5 VH variable genes

				10	20	30	40	50	
(15)	2d12.5	VH native hybridoma	1	GTGAAGCTGCAGGAGTCAGGACCTGGCCTAGTGCAGCCCTCACAGAGCCT	50				
(16)	2d12.5	VH native cloned	1	.....T.....	50				
(17)	2d12.5	VH N87D_cloned	1	.....T.....	50				
(18)	2d12.5	VH N87D_G53C_cloned	1	.....T.....	50				
(19)	2d12.5	VH_N87D_G54C_cloned	1	.....T..G.....	50				
(20)	2d12.5	VH N87D_G55C_cloned	1	.....T.....	50				
				60	70	80	90	100	
	2d12.5	VH native hybridoma	51	GTCCATCACCTGCACGGTCTCTGGTTTCTCATTAACTGACTATGGTGTAC	100				
	2d12.5	VH native cloned	51	.....	100				
	2d12.5	VH N87D_cloned	51	.....	100				
	2d12.5	VH N87D_G53C_cloned	51	.....	100				
	2d12.5	VH_N87D_G54C_cloned	51	.....	100				
	2d12.5	VH N87D_G55C_cloned	51	.....	100				
				110	120	130	140	150	
	2d12.5	VH native hybridoma	101	ACTGGGTTTCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGAGTGATA	150				
	2d12.5	VH native cloned	101	.....	150				
	2d12.5	VH N87D_cloned	101	.....	150				
	2d12.5	VH N87D_G53C_cloned	101	.....	150				
	2d12.5	VH_N87D_G54C_cloned	101	.....	150				
	2d12.5	VH N87D_G55C_cloned	101	.....	150				
				160	170	180	190	200	
	2d12.5	VH native hybridoma	151	TGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTTCATATCCAGACTGAA	200				
	2d12.5	VH native cloned	151	.....	200				
	2d12.5	VH N87D_cloned	151	.....	200				
	2d12.5	VH N87D_G53C_cloned	151	.....T.....	200				
	2d12.5	VH_N87D_G54C_cloned	151	.....T.T.....	200				
	2d12.5	VH N87D_G55C_cloned	151	.....T.....	200				
				210	220	230	240	250	
	2d12.5	VH native hybridoma	201	CATCTACAAGGACAATTCGAAGAACCAAGTTTTCTTTGAAATGAACAGTC	250				
	2d12.5	VH native cloned	201	.....	250				
	2d12.5	VH N87D_cloned	201	.....	250				
	2d12.5	VH N87D_G53C_cloned	201	.....	250				
	2d12.5	VH_N87D_G54C_cloned	201	.....	250				
	2d12.5	VH N87D_G55C_cloned	201	.....	250				
				260	270	280	290	300	
	2d12.5	VH native hybridoma	251	TGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGTAGCTAC	300				
	2d12.5	VH native cloned	251	.....	300				
	2d12.5	VH N87D_cloned	251	.....G.....	300				
	2d12.5	VH N87D_G53C_cloned	251	.....G.....	300				
	2d12.5	VH_N87D_G54C_cloned	251	.....G.....	300				
	2d12.5	VH N87D_G55C_cloned	251	.....G.....	300				
				310	320	330	340	350	
	2d12.5	VH native hybridoma	301	CCTTACAACCTACTTCGATGTCTGGGGCCAAGGGACCACAGTCACCGTCTC	350				
	2d12.5	VH native cloned	301	.....G.....	350				
	2d12.5	VH N87D_cloned	301	.....G.....	350				
	2d12.5	VH N87D_G53C_cl n d	301	.....G.....	350				
	2d12.5	VH_N87D_G54C_clon d	301	.....G.....	350				
	2d12.5	VH N87D_G55C_clon d	301	.....G.....	350				

FIG. 3B

2D12.5 VH variable genes

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      ....
2d12.5 VH native hybridoma 351 CTCA 354
2d12.5 VH native cloned   351 .G.. 354
2d12.5 VH N87D_cloned     351 .G.. 354
2d12.5 VH N87D_G53C_cloned 351 .G.. 354
2d12.5 VH_N87D_G54C_cloned 351 .G.. 354
2d12.5 VH N87D_G55C_cloned 351 .G.. 354
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FIG. 4

## Translation of 2D12.5 VL genes

			10	20	30	40	50	
(21) 2d12.5 VL native hybridoma	1	AVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIG						50
(22) 2d12.5 VL native cloned	1	.....						50
(23) 2d12.5 VL N53C_cloned	1	.....						50
			60	70	80	90	100	
2d12.5 VL native hybridoma	51	GNNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWVFG						100
2d12.5 VL native cloned	51	.....						100
2d12.5 VL N53C_cloned	51	.C.....						100
		.... ....						
2d12.5 VL native hybridoma	101	GGTRLTVLG						109
2d12.5 VL native cloned	101	...K....S						109
2d12.5 VL N53C_cloned	101	...K....S						109

## FIG. 5

				10	20	30	40	50	
(24)	2d12.5 VL native hybridoma	1	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						
(25)	2d12.5 VL native cloned	1	GCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGT						50
(26)	2d12.5 VL N53C_cloned	1	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						50
				60	70	80	90	100	
	2d12.5 VL native hybridoma	51	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						
	2d12.5 VL native cloned	51	CACACTCACTTGTCTGCTCAAGTACTGGGGCTGTTACGACTAGTAACATATG						100
	2d12.5 VL N53C_cloned	51	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						100
				110	120	130	140	150	
	2d12.5 VL native hybridoma	101	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						
	2d12.5 VL native cloned	101	CCAAGTGGGTCCTCAAGAGAAACCAGATCATTATTCACCTGGTCTAATAGGT						150
	2d12.5 VL N53C_cloned	101	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						150
				160	170	180	190	200	
	2d12.5 VL native hybridoma	151	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						
	2d12.5 VL native cloned	151	GGTAATAATAACCGACCTCCAGGTGTTCTCTGCCAGATTCTCAGGCTCCCT						200
	2d12.5 VL N53C_cloned	151	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						200
			...TG..... ..... ..... ..... ..... ..... ..... ..... ..... .....						200
				210	220	230	240	250	
	2d12.5 VL native hybridoma	201	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						
	2d12.5 VL native cloned	201	GATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTGAGGATG						250
	2d12.5 VL N53C_cloned	201	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						250
				260	270	280	290	300	
	2d12.5 VL native hybridoma	251	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						
	2d12.5 VL native cloned	251	AGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTGTTTCGGT						300
	2d12.5 VL N53C_cloned	251	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						300
				310	320				
	2d12.5 VL native hybridoma	301	..... ..... ..... ..... ..... ..... ..... ..... ..... .....						
	2d12.5 VL native cloned	301	GGAGGAACCAGACTGACTGTCCTAGGC						327
	2d12.5 VL N53C cloned	301	..G.....A..... ..... ..... ..... ..... ..... ..... ..... ..... .....						327
			..G.....A..... ..... ..... ..... ..... ..... ..... ..... ..... .....						327

FIG. 6

## Translation of Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

			10	20	30	40	50	
(27)	2dVL-TTCL native_cloned	1	..... ..... ..... ..... ..... ..... ..... .....					
(28)	2dVL-TTCL N53C_cloned	1	RSVVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGL					50
(29)	2d12.5 VL native hybridoma	1	..... ..... ..... ..... ..... ..... ..... .....					50
(30)	TTCL template for gene assembly	1	----- ----- ----- ----- ----- ----- ----- -----					50
			60	70	80	90	100	
	2dVL-TTCL native_cloned	51	IGGNNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWV					101
	2dVL-TTCL N53C_cloned	51	...C..... ..... ..... ..... ..... ..... ..... .....					101
	2d12.5 VL native hybridoma	49	..... ..... ..... ..... ..... ..... ..... .....					99
	TTCL template for gene assembly	1	----- ----- ----- ----- ----- ----- ----- -----					1
			110	120	130	140	150	
	2dVL-TTCL native_cloned	101	FGGGTKLTVLSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV					151
	2dVL-TTCL N53C_cloned	101	..... ..... ..... ..... ..... ..... ..... .....					151
	2d12.5 VL native hybridoma	99	.....R.....G..... ..... ..... ..... ..... ..... ..... .....					101
	TTCL template for gene assembly	1	----- ----- ----- ----- ----- ----- ----- -----					99
			160	170	180	190	200	
	2dVL-TTCL native_cloned	151	QWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV					201
	2dVL-TTCL N53C_cloned	151	..... ..... ..... ..... ..... ..... ..... .....					201
	2d12.5 VL native hybridoma		..... ..... ..... ..... ..... ..... ..... .....					
	TTCL template for gene assembly	40	----- ----- ----- ----- ----- ----- ----- -----					89
			210	220				
	2dVL-TTCL native_cloned	201	THQGLSLPVTKSFNRGEC*F*	221				
	2dVL-TTCL N53C_cloned	201	..... ..... ..... ..... ..... ..... ..... .....					221
	2d12.5 VL native hybridoma		..... ..... ..... ..... ..... ..... ..... .....					
	TTCL template for gene assembly	90	----- ----- ----- ----- ----- ----- ----- -----					107

FIG. 7A

## Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

		10	20	30	40	50	
(31)	2dVL-TTCL native_cloned	1	AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGA	50			
(32)	2dVL-TTCL N53C_cloned	1	.....	50			
(33)	2d12.5 VL native hybridoma	1	-----	44			
(34)	TTCL template for gene assem	1	-----	1			
		60	70	80	90	100	
	2dVL-TTCL native_cloned	51	AACAGTCACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACGACTAGTA	100			
	2dVL-TTCL N53C_cloned	51	.....	100			
	2d12.5 VL native hybridoma	45	.....	94			
	TTCL template for gene assem	1	-----	1			
		110	120	130	140	150	
	2dVL-TTCL native_cloned	101	ACTATGCCAACTGGGTCCAAGAGAAACCAGATCATTATTCACTGGTCTA	150			
	2dVL-TTCL N53C_cloned	101	.....	150			
	2d12.5 VL native hybridoma	95	.....	144			
	TTCL template for gene assem	1	-----	1			
		160	170	180	190	200	
	2dVL-TTCL native_cloned	151	ATAGGTGGTAATAATAACCGACCTCCAGGTGTTCTGCCAGATTCTCAGG	200			
	2dVL-TTCL N53C_cloned	151	.....TG.....	200			
	2d12.5 VL native hybridoma	145	.....	194			
	TTCL template for gene assem	1	-----	1			
		210	220	230	240	250	
	2dVL-TTCL native_cloned	201	CTCCCTGATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTG	250			
	2dVL-TTCL N53C_cloned	201	.....	250			
	2d12.5 VL native hybridoma	195	.....	244			
	TTCL template for gene assem	1	-----	1			
		260	270	280	290	300	
	2dVL-TTCL native_cloned	251	AGGATGAGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGGTG	300			
	2dVL-TTCL N53C_cloned	251	.....	300			
	2d12.5 VL native hybridoma	245	.....	294			
	TTCL template for gene assem	1	-----	1			
		310	320	330	340	350	
	2dVL-TTCL native_cloned	301	TTCGGTGGGGGAACCAAAGTACTGTCTAAGCCGAAGTGTGGCTGCACC	350			
	2dVL-TTCL N53C_cloned	301	.....	350			
	2d12.5 VL native hybridoma	295	.....A.....G.....G..	327			
	TTCL template for gene assem	1	-----	17			
		360	370	380	390	400	
	2dVL-TTCL native_cloned	351	ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGT	400			
	2dVL-TTCL N53C_cloned	351	.....	400			
	2d12.5 VL native hybridoma		.....				
	TTCL template for gene assem	18	.....	67			
		410	420	430	440	450	
	2dVL-TTCL native_cloned	401	CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTA	450			
	2dVL-TTCL N53C_cloned	401	.....	450			
	2d12.5 VL native hybridoma		.....				
	TTCL template for gene assem	68	.....	117			



FIG. 7B

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

		460	470	480	490	500	
2dVL-TTCL native_cloned	451	..... ..... ..... ..... ..... .....	CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT	500			
2dVL-TTCL N53C_cloned	451	..... ..... ..... ..... ..... .....	.....	500			
2d12.5 VL native hybridoma							
TTCL template for gene assem	118	..... ..... ..... ..... ..... .....	.....	167			
		510	520	530	540	550	
2dVL-TTCL native_cloned	501	..... ..... ..... ..... ..... .....	CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGA	550			
2dVL-TTCL N53C_cloned	501	..... ..... ..... ..... ..... .....	.....	550			
2d12.5 VL native hybridoma							
TTCL template for gene assem	168	..... ..... ..... ..... ..... .....	.....	217			
		560	570	580	590	600	
2dVL-TTCL native_cloned	551	..... ..... ..... ..... ..... .....	CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC	600			
2dVL-TTCL N53C_cloned	551	..... ..... ..... ..... ..... .....	.....	600			
2d12.5 VL native hybridoma							
TTCL template for gene assem	218	..... ..... ..... ..... ..... .....	.....	267			
		610	620	630	640	650	
2dVL-TTCL native_cloned	601	..... ..... ..... ..... ..... .....	ACCCATCAGGGCCTGAGCTTGCCCGTCACAAAGAGCTTCAACAGGGGAGA	650			
2dVL-TTCL N53C_cloned	601	..... ..... ..... ..... ..... .....	.....T.....	650			
2d12.5 VL native hybridoma							
TTCL template for gene assem	268	..... ..... ..... ..... ..... .....	.....	317			
		660					
2dVL-TTCL native_cloned	651	..... ..... .....	GTGTTAATTCTAGA	664			
2dVL-TTCL N53C_cloned	651	..... ..... .....	.....	664			
2d12.5 VL native hybridoma							
TTCL template for gene assem	318	..... ..... .....	.....	322			

FIG. 8

## Translation of Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

			10	20	30	40	50	
(35) 2dVH-TTCH_native cloned	1	RSVKLQESGPGLVQPSQSL	SITCTVSGFSLTDYGVH	WVRQSPGKGLEWLG				50
(36) 2dVH-TTCH_N87D_clon d	1	.....	.....	.....	.....	.....	.....	50
(37) 2dVH-TTCH_N87D_G53C_cloned	1	.....	.....	.....	.....	.....	.....	50
(38) 2dVH-TTCH_N87D_G54C_cloned	1	.....	.....	.....	.....	.....	.....	50
(39) 2dVH-TTCH_N87D_G55C_cloned	1	.....	.....	.....	.....	.....	.....	50
(40) 2dVH-TTCH expected sequence	1	.....	.....	.....	.....	.....	.....	50
(41) 2d12.5 VH native hybridoma	1	.....	.....	.....	.....	.....	.....	48
			60	70	80	90	100	
2dVH-TTCH_native cloned	51	VIWSGGGTAYTA	AFISRLNIYKD	NSKNQVFFEM	NSLQANDTAM	YYCARRG		100
2dVH-TTCH_N87D_cloned	51	.....	.....	.....	.....	.....	.....	100
2dVH-TTCH_N87D_G53C_cloned	51	.....C.....	.....	.....	.....	.....	.....	100
2dVH-TTCH_N87D_G54C_cloned	51	.....C.....	.....	.....	.....	.....	.....	100
2dVH-TTCH_N87D_G55C_cloned	51	.....C.....	.....	.....	.....	.....	.....	100
2dVH-TTCH expected sequence	51	.....	.....	.....	.....	.....	.....	100
2d12.5 VH native hybridoma	49	.....	.....	.....	.....	.....	.....	98
			110	120	130	140	150	
2dVH-TTCH_native cloned	101	SYPPYNYFDVW	GQTTVTVSA	AASKGPSV	FPLAPSSK	STSGGTAAL	GCLVK	150
2dVH-TTCH_N87D_cloned	101	.....	.....	.....	.....	.....	.....	150
2dVH-TTCH_N87D_G53C_cloned	101	.....	.....	.....	.....	.....	.....	150
2dVH-TTCH_N87D_G54C_cloned	101	.....	.....	.....	.....	.....	.....	150
2dVH-TTCH_N87D_G55C_cloned	101	.....	.....	.....	.....	.....	.....	150
2dVH-TTCH expected sequence	101	.....	.....	.....	.....	.....	.....	150
2d12.5 VH native hybridoma	99	.....	.....	.....S	.....	.....	.....	118
			160	170	180	190	200	
2dVH-TTCH_native cloned	151	DYFPEPVTV	SWNSGALT	SGVHTFPA	VLQSSGLY	SLSSVTV	PSSSLGTQT	200
2dVH-TTCH_N87D_cloned	151	.....	.....	.....	.....	.....	.....	200
2dVH-TTCH_N87D_G53C_cloned	151	.....	.....	.....	.....	.....	.....	200
2dVH-TTCH_N87D_G54C_cloned	151	.....	.....	.....	.....	.....	.....	200
2dVH-TTCH_N87D_G55C_cloned	151	.....	.....	.....	.....	.....	.....	200
2dVH-TTCH expected sequence	151	.....	.....	.....	.....	.....	.....	200
2d12.5 VH native hybridoma		.....	.....	.....	.....	.....	.....	
			210	220				
2dVH-TTCH_native cloned	201	YICNVNHKPS	NTKVDRK	AEPRKSCDKSR				227
2dVH-TTCH_N87D_cloned	201	.....	.....	.....	.....	.....	.....	227
2dVH-TTCH_N87D_G53C_cloned	201	.....	.....	.....	.....	.....	.....	227
2dVH-TTCH_N87D_G54C_cloned	201	.....	.....	.....	.....	.....	.....	227
2dVH-TTCH_N87D_G55C_cloned	201	.....	.....	.....	.....	.....	.....	227
2dVH-TTCH expected sequence	201	.....	.....	.....	.....	.....	.....	227
2d12.5 VH native hybridoma		.....	.....	.....	.....	.....	.....	

FIG. 9A

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

		10	20	30	40	50	
(42) 2dVH-TTCH_native cloned	1	AGATCTGTGAAGCTGCAGGAGTCTGGACCTGGCCTAGTGCAGCCCTCACA	50				
(43) 2dVH-TTCH_N87D_cloned	1	.....	50				
(44) 2dVH-TTCH_N87D_G53C_cloned	1	.....	50				
(45) 2dVH-TTCH_N87D_G54C_cloned	1	.....G.....	50				
(46) 2dVH-TTCH_N87D_G55C_cloned	1	.....	50				
(47) 2dVH-TTCH expected sequence	1	.....	50				
(48) 2d12.5 VH native hybridoma	1	.....A.....	44				
		60	70	80	90	100	
2dVH-TTCH_native cloned	51	GAGCCTGTCCATCACCTGCACGGTCTCTGGTTTCTCATTAACTGACTATG	100				
2dVH-TTCH_N87D_cloned	51	.....	100				
2dVH-TTCH_N87D_G53C_cloned	51	.....	100				
2dVH-TTCH_N87D_G54C_cloned	51	.....	100				
2dVH-TTCH_N87D_G55C_cloned	51	.....	100				
2dVH-TTCH expected sequence	51	.....	100				
2d12.5 VH native hybridoma	45	.....	94				
		110	120	130	140	150	
2dVH-TTCH_native cloned	101	GTGTACACTGGGTTTCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGA	150				
2dVH-TTCH_N87D_cloned	101	.....	150				
2dVH-TTCH_N87D_G53C_cloned	101	.....	150				
2dVH-TTCH_N87D_G54C_cloned	101	.....	150				
2dVH-TTCH_N87D_G55C_cloned	101	.....	150				
2dVH-TTCH expected sequence	101	.....	150				
2d12.5 VH native hybridoma	95	.....	144				
		160	170	180	190	200	
2dVH-TTCH_native cloned	151	GTGATATGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTCATATCCAG	200				
2dVH-TTCH_N87D_cloned	151	.....	200				
2dVH-TTCH_N87D_G53C_cloned	151	.....T.....	200				
2dVH-TTCH_N87D_G54C_cloned	151	.....T.T.....	200				
2dVH-TTCH_N87D_G55C_cloned	151	.....T.....	200				
2dVH-TTCH expected sequence	151	.....	200				
2d12.5 VH native hybridoma	145	.....	194				
		210	220	230	240	250	
2dVH-TTCH_native cloned	201	ACTGAACATCTACAAGGACAATTCCAAGAACCAAGTTTCTTTGAAATGA	250				
2dVH-TTCH_N87D_cloned	201	.....	250				
2dVH-TTCH_N87D_G53C_cloned	201	.....	250				
2dVH-TTCH_N87D_G54C_cloned	201	.....	250				
2dVH-TTCH_N87D_G55C_cloned	201	.....	250				
2dVH-TTCH expected sequence	201	.....	250				
2d12.5 VH native hybridoma	195	.....	244				
		260	270	280	290	300	
2dVH-TTCH_native cloned	251	ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT	300				
2dVH-TTCH_N87D_cloned	251	.....G.....	300				
2dVH-TTCH_N87D_G53C_cloned	251	.....G.....	300				
2dVH-TTCH_N87D_G54C_cloned	251	.....G.....	300				
2dVH-TTCH_N87D_G55C_cloned	251	.....G.....	300				
2dVH-TTCH expected sequence	251	.....	300				
2d12.5 VH native hybridoma	245	.....	294				
		310	320	330	340	350	
		.....					

FIG. 9B

## Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH_native cl ned	301	AGCTACCCCTTACAACCTACTTCGATGTCTGGGGCCAAGGGACCACGGTCAC	350
2dVH-TTCH_N87D_cl ned	301	.....	350
2dVH-TTCH_N87D_G53C_cloned	301	.....	350
2dVH-TTCH_N87D_G54C_cloned	301	.....	350
2dVH-TTCH_N87D_G55C_cloned	301	.....	350
2dVH-TTCH expected sequence	301	.....	350
2d12.5 VH native hybridoma	295	.....A.....	344
<div> <div>360370380390400</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div>			
2dVH-TTCH_native cloned	351	CGTCTCCGCAGCCTCCACCAAGGGGCCCATCGGTCTTCCCCCTGGCACCCT	400
2dVH-TTCH_N87D_cloned	351	.....	400
2dVH-TTCH_N87D_G53C_cloned	351	.....	400
2dVH-TTCH_N87D_G54C_cloned	351	.....	400
2dVH-TTCH_N87D_G55C_cloned	351	.....	400
2dVH-TTCH expected sequence	351	.....	400
2d12.5 VH native hybridoma	345	.....T..	354
<div> <div>410420430440450</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div>			
2dVH-TTCH_native cloned	401	CCTCCAAGAGCACCTCTGGGGGACAGCGGCCCTGGGCTGCCTGGTCAAG	450
2dVH-TTCH_N87D_cloned	401	.....	450
2dVH-TTCH_N87D_G53C_cloned	401	.....	450
2dVH-TTCH_N87D_G54C_cloned	401	.....	450
2dVH-TTCH_N87D_G55C_cloned	401	.....	450
2dVH-TTCH expected sequence	401	.....	450
2d12.5 VH native hybridoma			
<div> <div>460470480490500</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div>			
2dVH-TTCH_native cloned	451	GACTACTTCCCCGAACCGGTGACGGTGTCTTGGAACCTCAGGCGCCCTGAC	500
2dVH-TTCH_N87D_cloned	451	.....	500
2dVH-TTCH_N87D_G53C_cloned	451	.....	500
2dVH-TTCH_N87D_G54C_cloned	451	.....	500
2dVH-TTCH_N87D_G55C_cloned	451	.....	500
2dVH-TTCH expected sequence	451	.....G.....	500
2d12.5 VH native hybridoma			
<div> <div>510520530540550</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div>			
2dVH-TTCH_native cloned	501	CAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACT	550
2dVH-TTCH_N87D_cloned	501	.....	550
2dVH-TTCH_N87D_G53C_cloned	501	.....	550
2dVH-TTCH_N87D_G54C_cloned	501	.....	550
2dVH-TTCH_N87D_G55C_cloned	501	.....	550
2dVH-TTCH expected sequence	501	.....	550
2d12.5 VH native hybridoma			
<div> <div>560570580590600</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div>			
2dVH-TTCH_native cloned	551	CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC	600
2dVH-TTCH_N87D_cloned	551	.....	600
2dVH-TTCH_N87D_G53C_cloned	551	.....	600
2dVH-TTCH_N87D_G54C_cloned	551	.....	600
2dVH-TTCH_N87D_G55C_cloned	551	.....	600
2dVH-TTCH expected sequence	551	.....	600
2d12.5 VH native hybridoma			
<div> <div>610620630640650</div> <div>..... ..... ..... ..... ..... ..... ..... ..... ..... ..... </div> </div>			
2dVH-TTCH_native cloned	601	TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA	650
2dVH-TTCH_N87D_cloned	601	.....	650

FIG. 9C

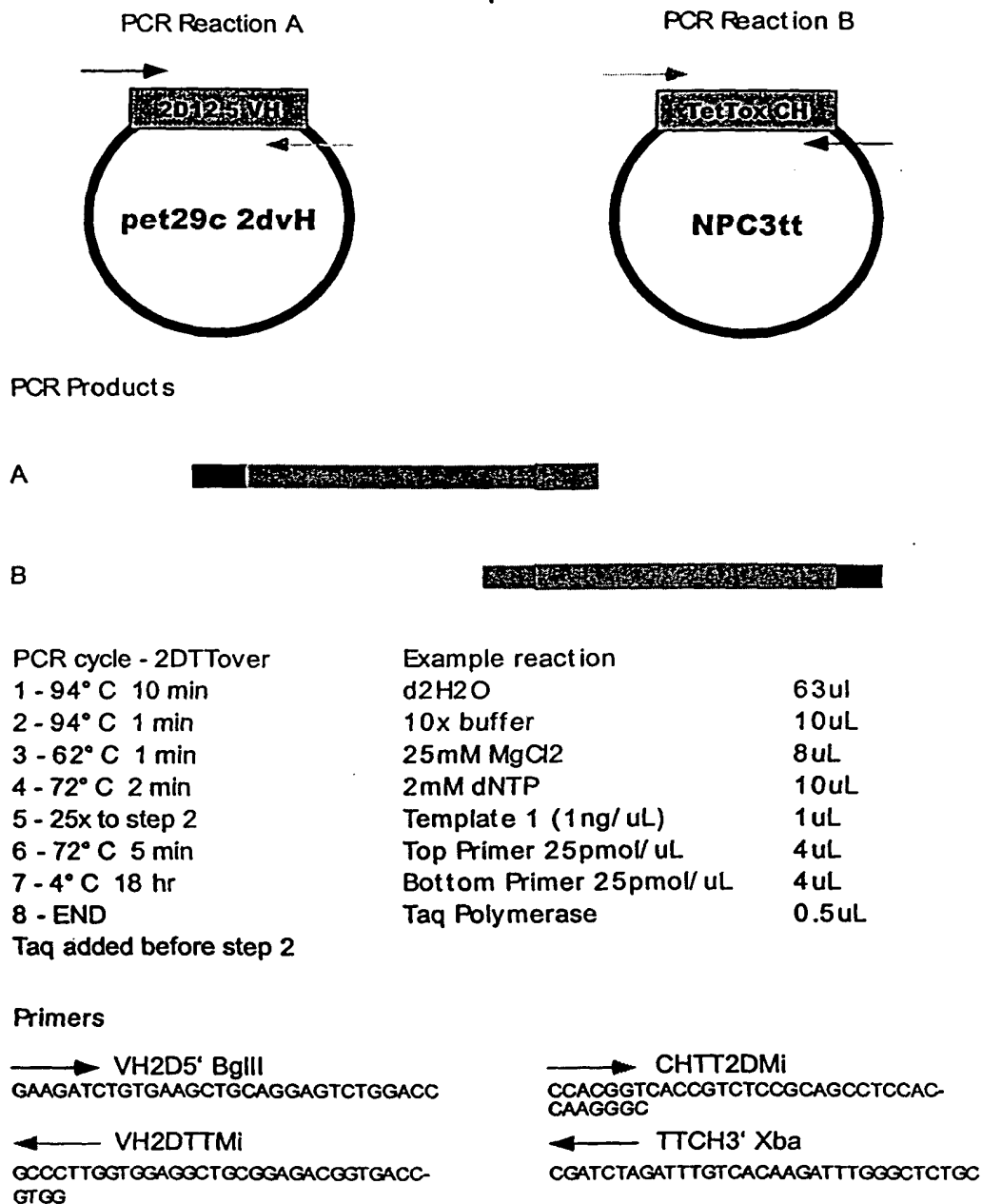
Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH N87D_G53C_cloned	601	.....	650
2dVH-TTCH N87D_G54C_cloned	601	.....	650
2dVH-TTCH N87D_G55C_cloned	601	.....	650
2dVH-TTCH expected sequence	601	.....	650
2d12.5 VH native hybridoma			

		660	670	680
		.... .... .... .... ....		
2dVH-TTCH_native cloned	651	AGCAGAGCCCAAATCTTGTGACAAATCTAGA	681	
2dVH-TTCH_N87D_cloned	651	.....	681	
2dVH-TTCH_N87D_G53C_cloned	651	.....	681	
2dVH-TTCH_N87D_G54C_cloned	651	.....	681	
2dVH-TTCH_N87D_G55C_cloned	651	.....	681	
2dVH-TTCH expected sequence	651	.....	681	
2d12.5 VH native hybridoma				

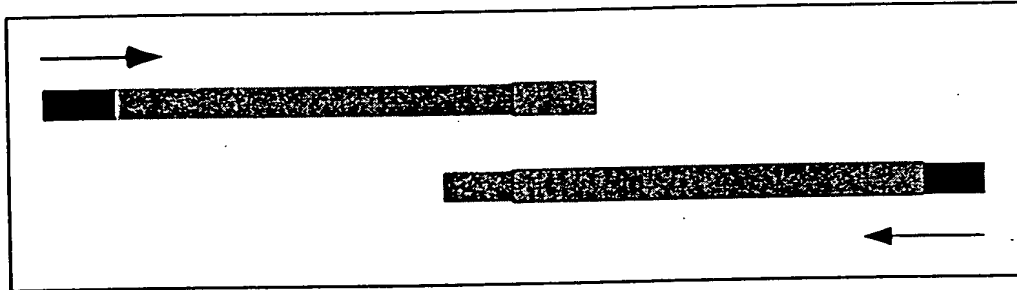
FIG. 10A

# Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 1



# Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 2

## PCR Reaction



### PCR cycle - 2DTTVent

- 1 - 95°C 10 min
  - 2 - 94°C 1 min
  - 3 - 60°C 1 min
  - 4 - 75°C 2 min
  - 5 - 4x to step 2
  - 6 - 94°C 1 min
  - 7 - 63°C 1 min
  - 8 - 75°C 2 min
  - 9 - 25x to step 6
  - 10 - 72°C 5 min
  - 11 - 4°C 18 hr
  - 12 - END
- Vent added before step 2  
Primers added before step 6

### Primers

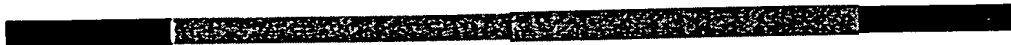
→ VH2D5' BglII  
GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← TTCH3' Xba  
CGATCTAGATTTGTCACAAGATTGGGCTCTGC

### Example reaction

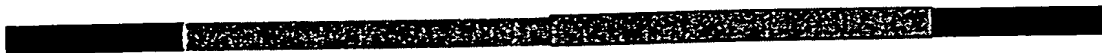
d2H2O	70uL
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template 1(1ng/ uL)	1uL
Template 2(1ng/ uL)	1uL
Top Primer 25pmol/ uL	4uL
Bottom Primer 25pmol/ uL	4uL
Vent Polymerase	0.5uL

### PCR Assembly Product

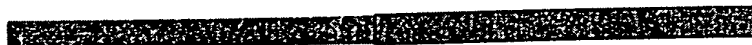


# Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His  
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

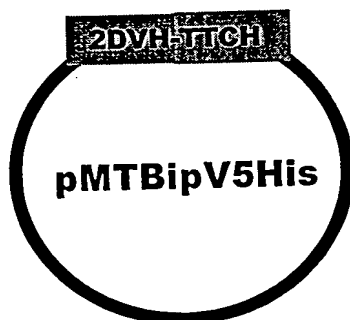
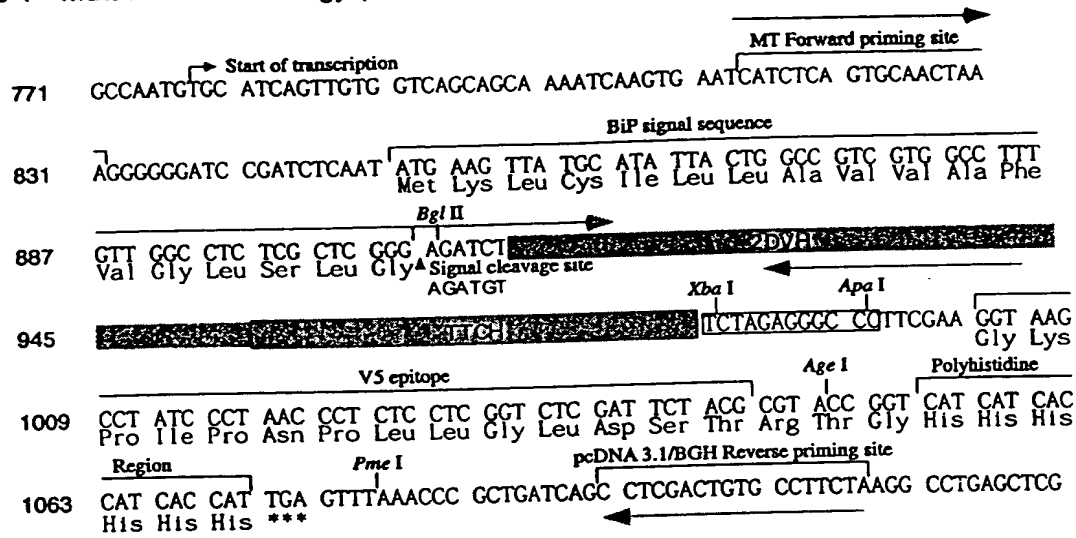


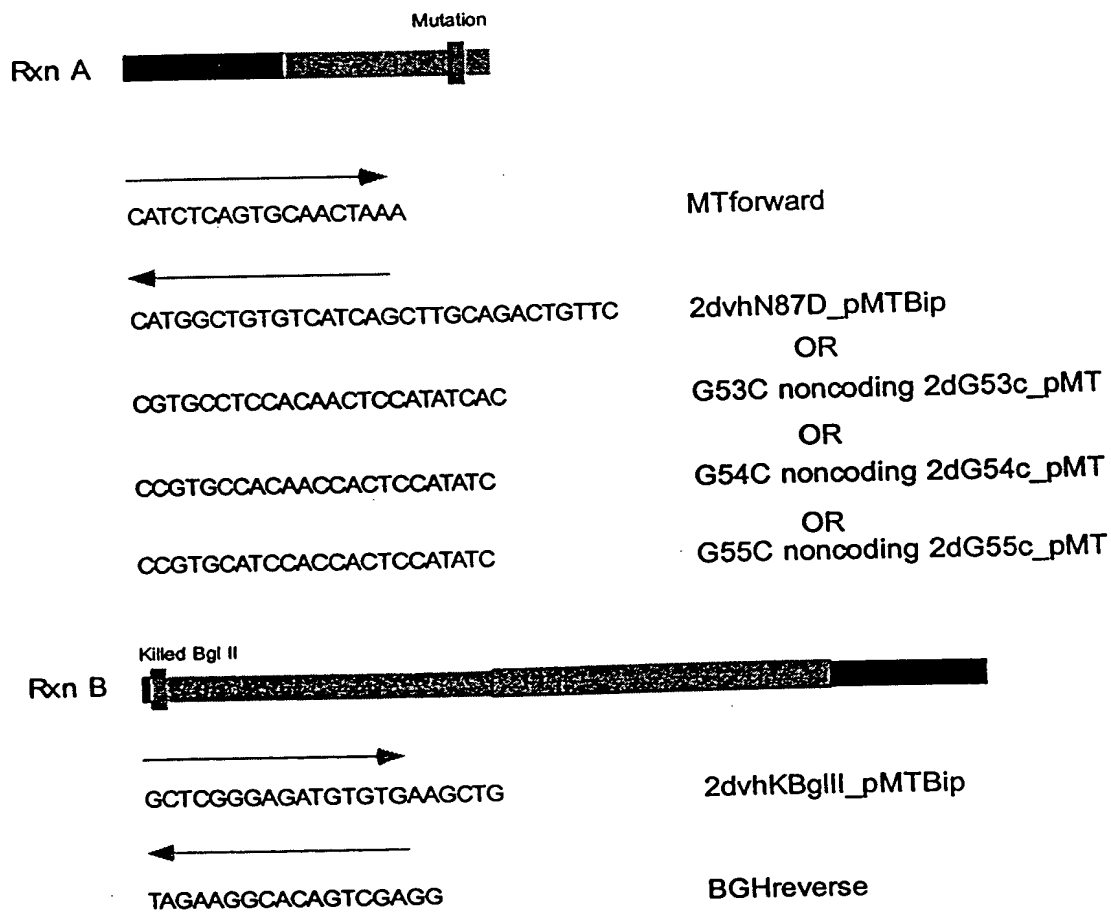


FIG. 10D

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



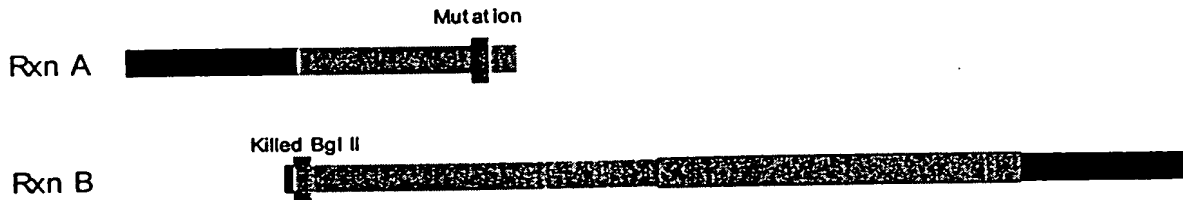
1st Set of PCR Reactions Producta A and B)



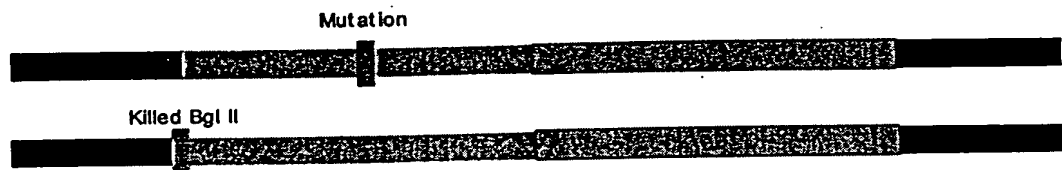
Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction (Mix Products of reaction A and B)

- 1) Extend
- 2) Amplify with outer primers (MTforward and BGHreverse)



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

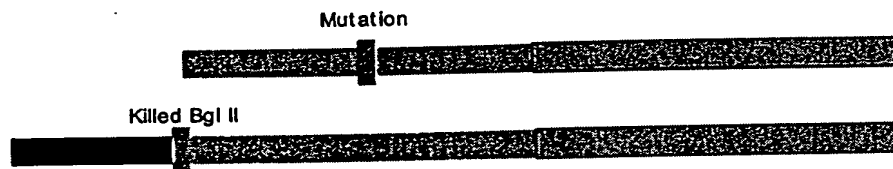


FIG. 10F

Step 1 - PCR Reaction MT-VENT

PCR cycle - MT-VENT

- 1 - 95° C 10 min
- 2 - 94° C 1 min
- 3 - 50° C 1 min
- 4 - 75° C 2 min
- 5 - 24x to step 2
- 6 - 75° C 5 min
- 7 - 4° C 18 hr
- 8 - END

VENT added before step 2

Primers added before step 1

Example reaction	
d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Vent Polymerase	0.5uL

Step 2 - PCR Reaction VHMUTTAQ

PCR cycle - VHMUTTAQ

- 1 - 95° C 10 min
- 2 - 94° C 1 min
- 3 - 68° C 1 min
- 4 - 72° C 2 min
- 5 - 4x to step 2
- 6 - 94° C 1 min
- 7 - 50° C 1 min
- 8 - 72° C 2 min
- 9 - 24x to step 6
- 10 - 72° C 5 min
- 11 - 4° C 18 hr
- 12 - END

Taq added before step 2

Primers added before step 6

Example reaction	
d2H2O	61ul
10x buffer	10uL
25mM MgCl2	8uL
2mM dNTP	10uL
Template 1 (1ng/uL)	1uL
Template 2 (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Taq Polymerase	0.5uL

FIG. 11A

# Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 1

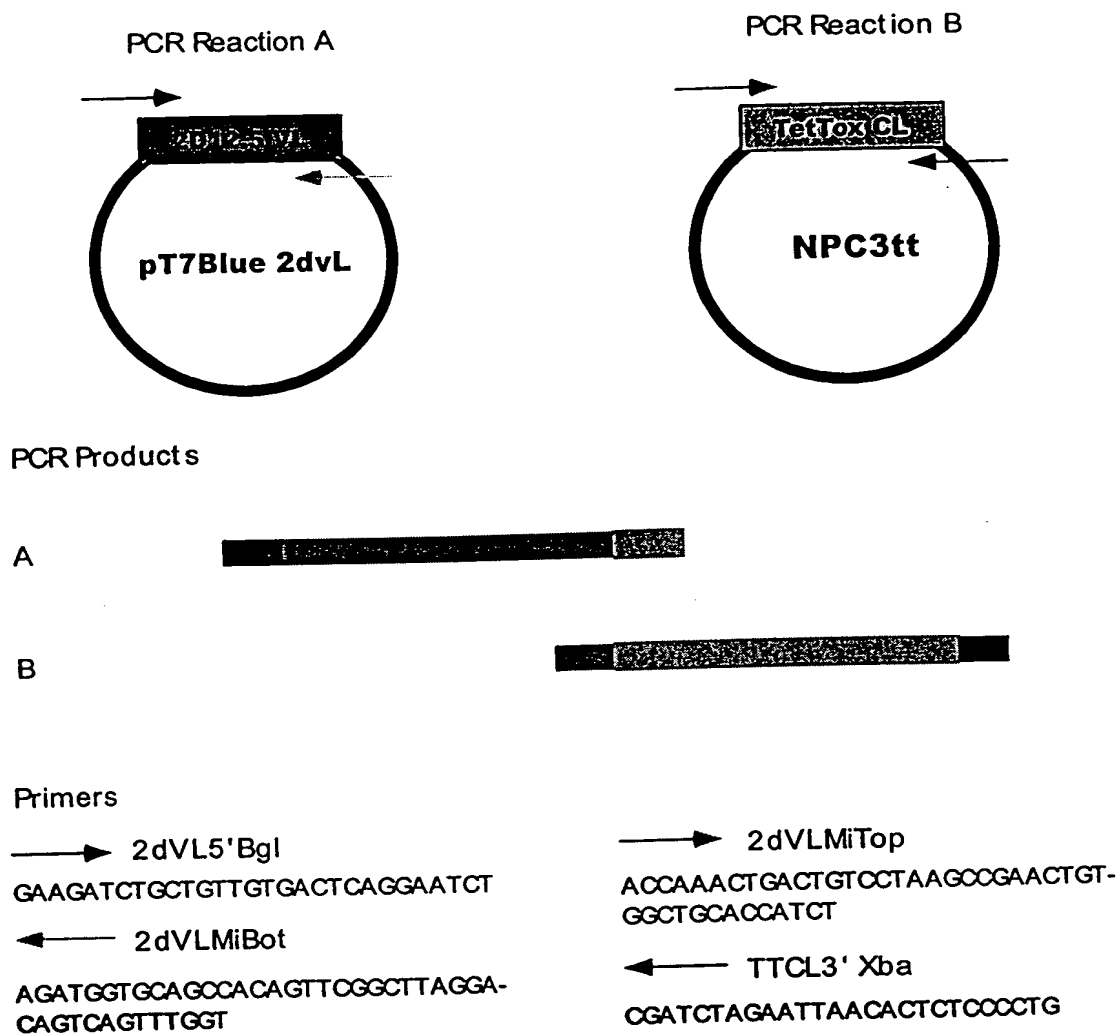
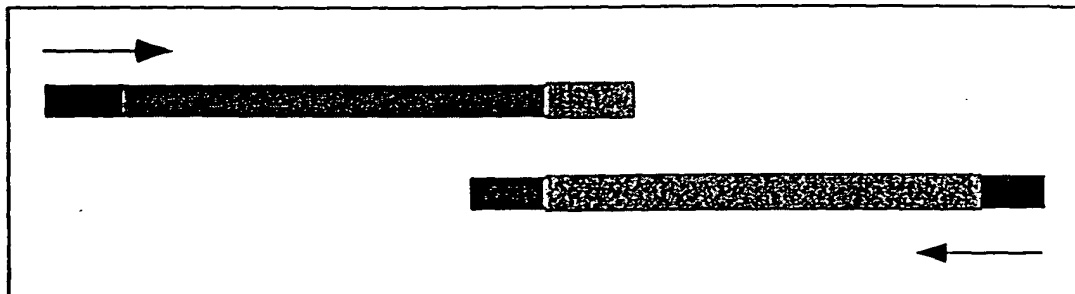


FIG. 11B

# Strategy for Assembly of Chimeric 2D12.5 Light Chain

## Step 2

### PCR Reaction



### Primers

→ 2dVL5' Bgl

GAAGATCTGCTGTTGTGACTCAGGAATCT

← TTCL3' Xba

CGATCTAGAATTAACACTCTCCCCTG

### PCR Assembly Product



# Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His  
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

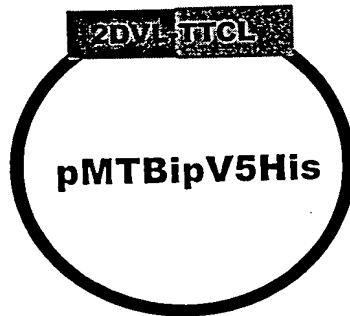
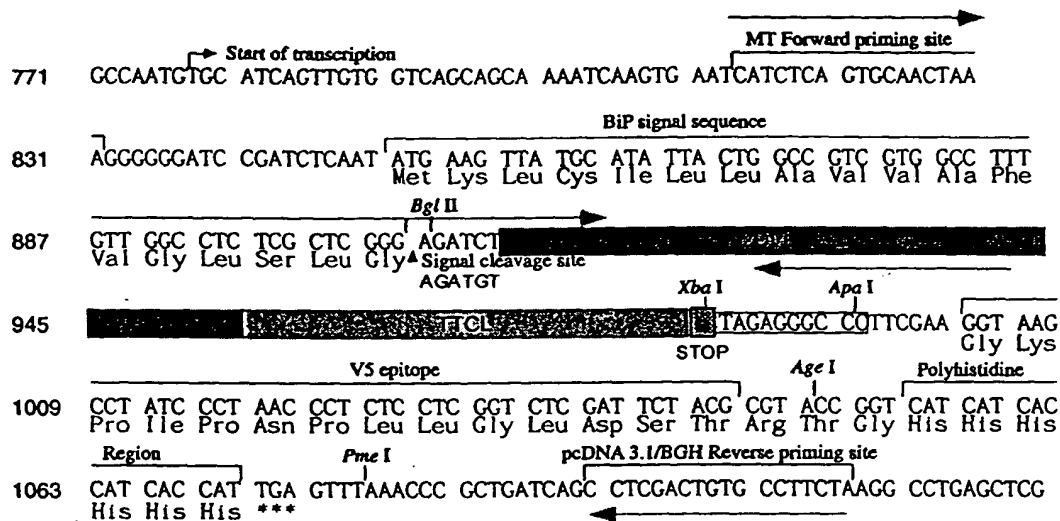


FIG. 11D

## Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



## 1st Set of PCR Reactions Producta A and B)

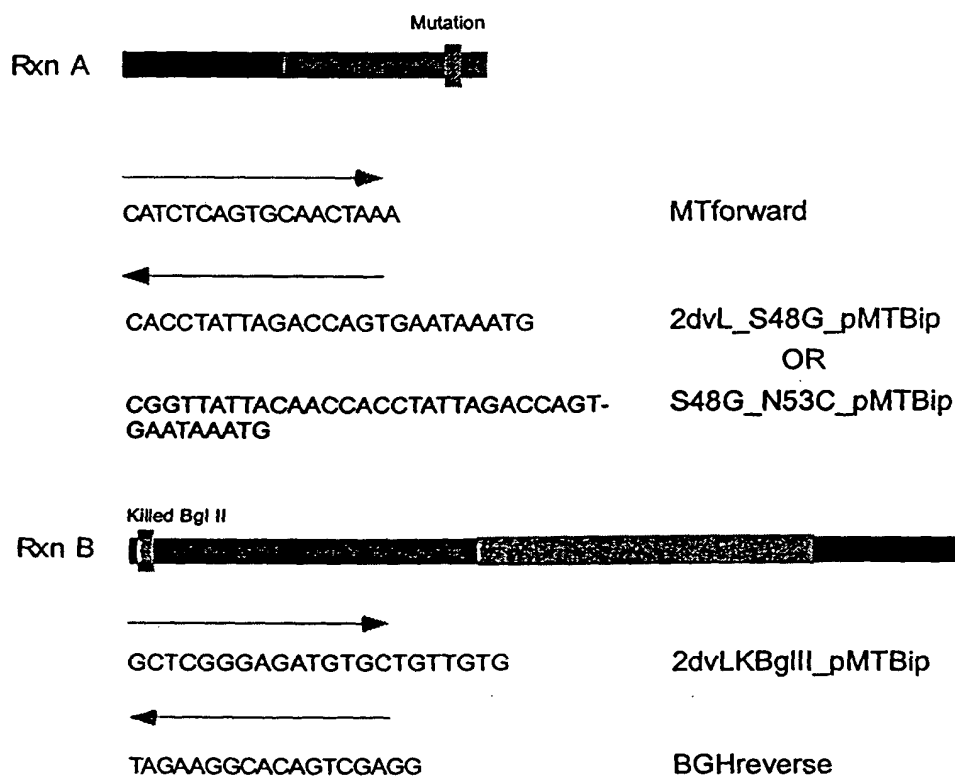


FIG. 11E

Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

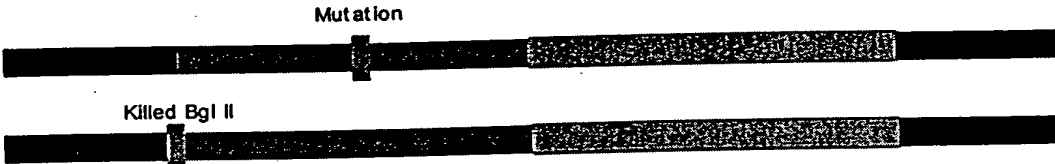
2nd PCR Reaction Mix Products of reaction B)

- 1) Extend
- 2) Amplify with outer primers (MTforward and BGHreverse)

Rxn A 

Rxn B 

2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

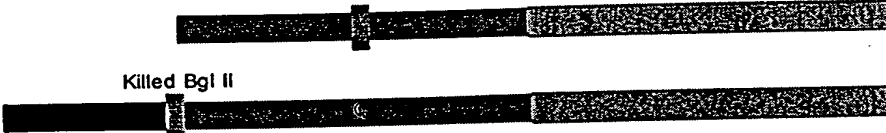




FIG. 11F

# Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 4

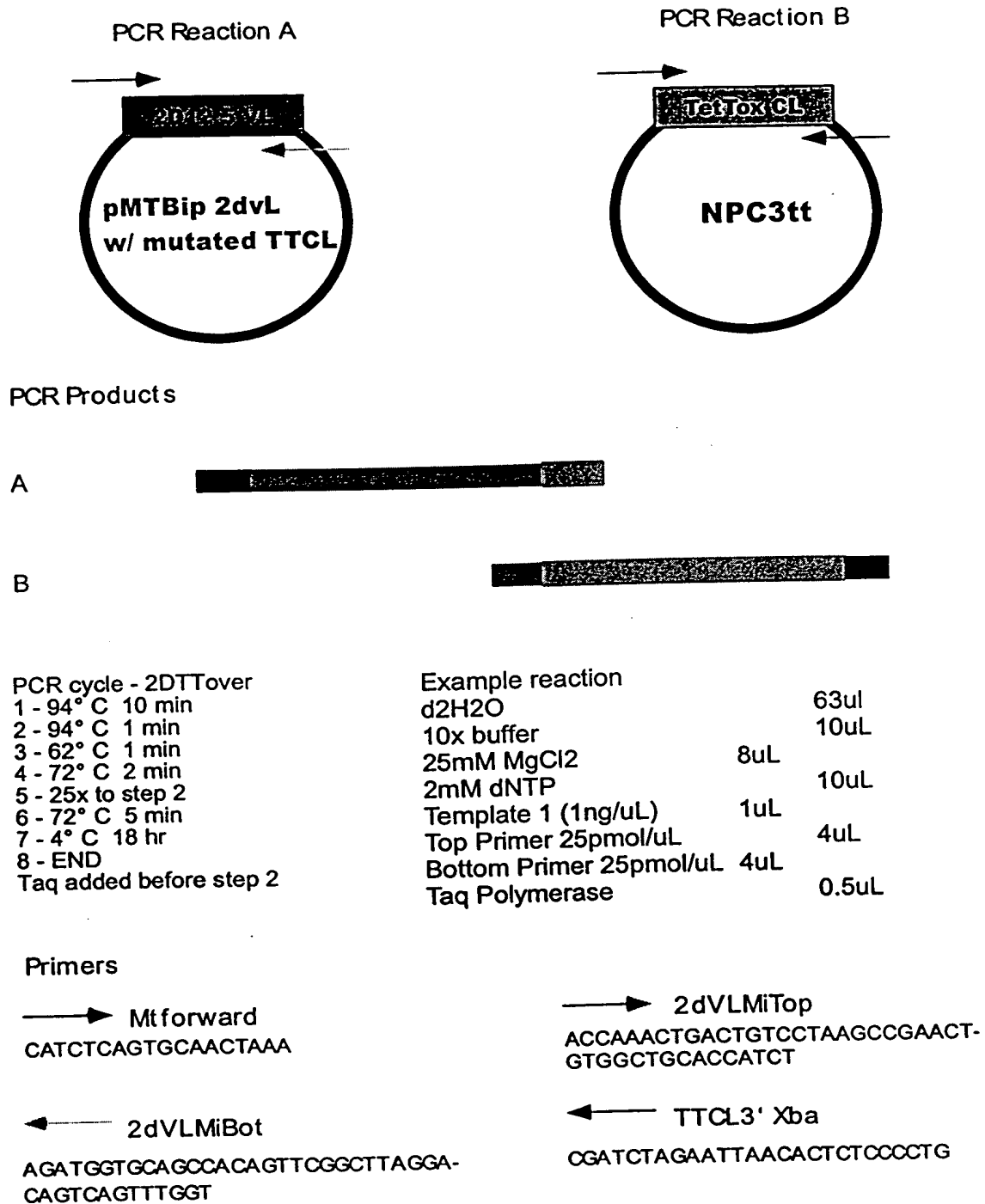


FIG. 11G

## Assembled Vectors for Transfection in S2 Cells

Each of the following has been cotransfected with the native light chain:

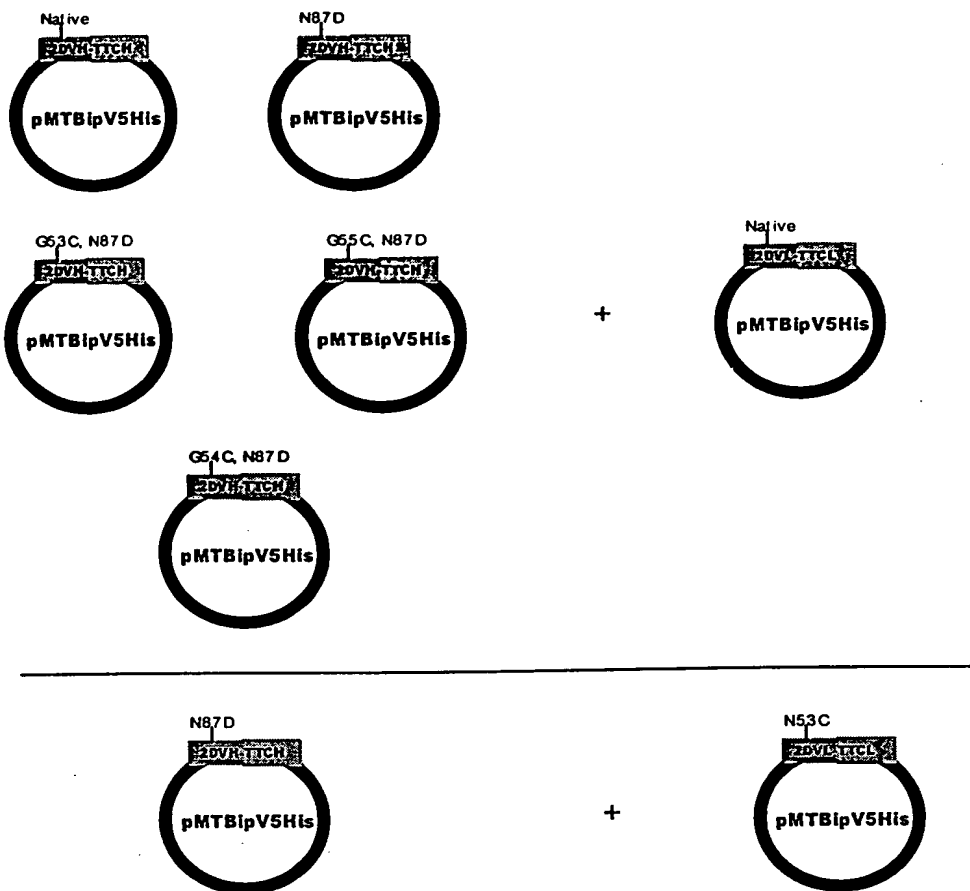


FIG. 12

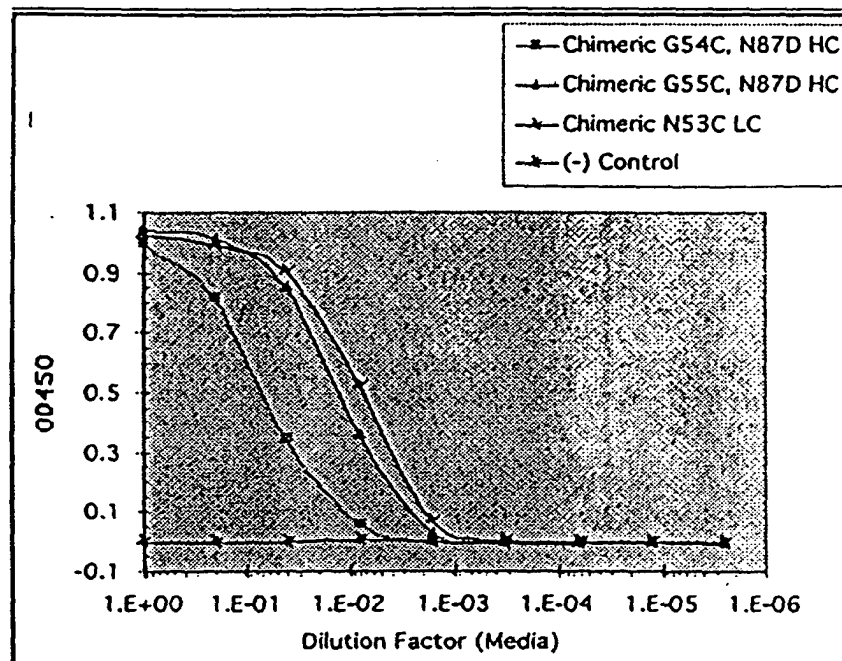
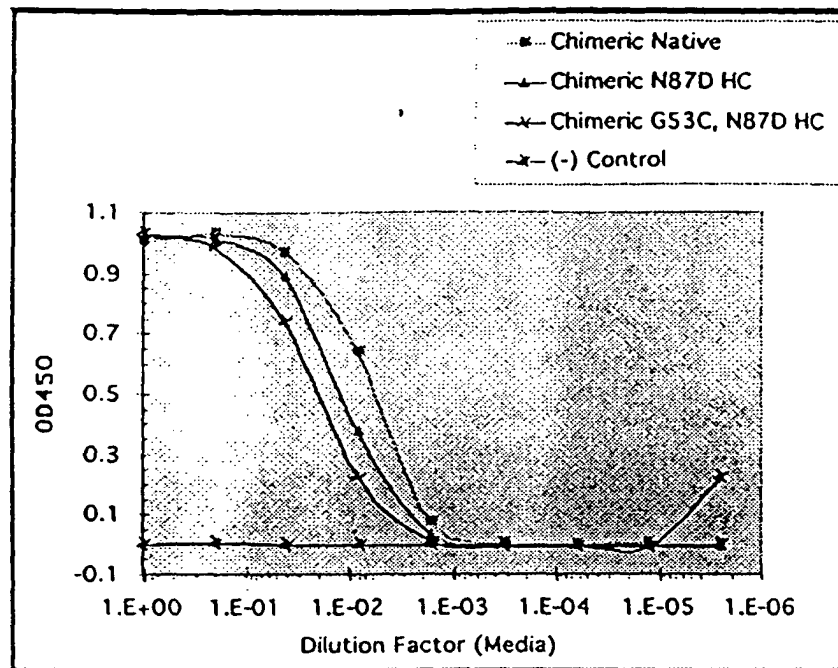


FIG. 13

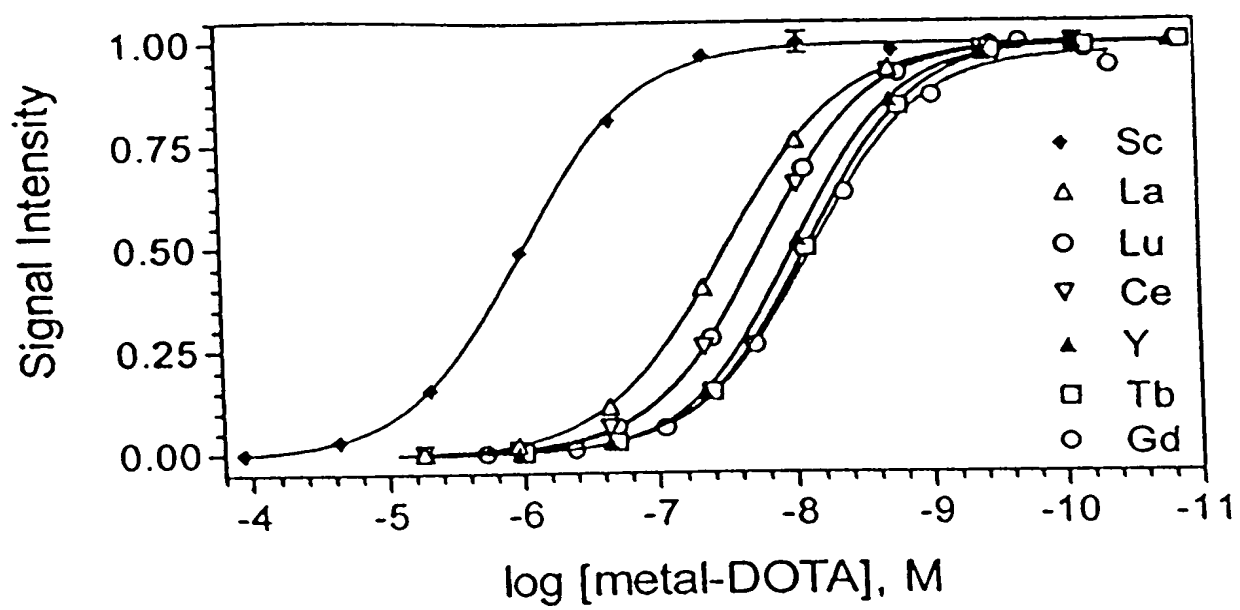


FIG. 14

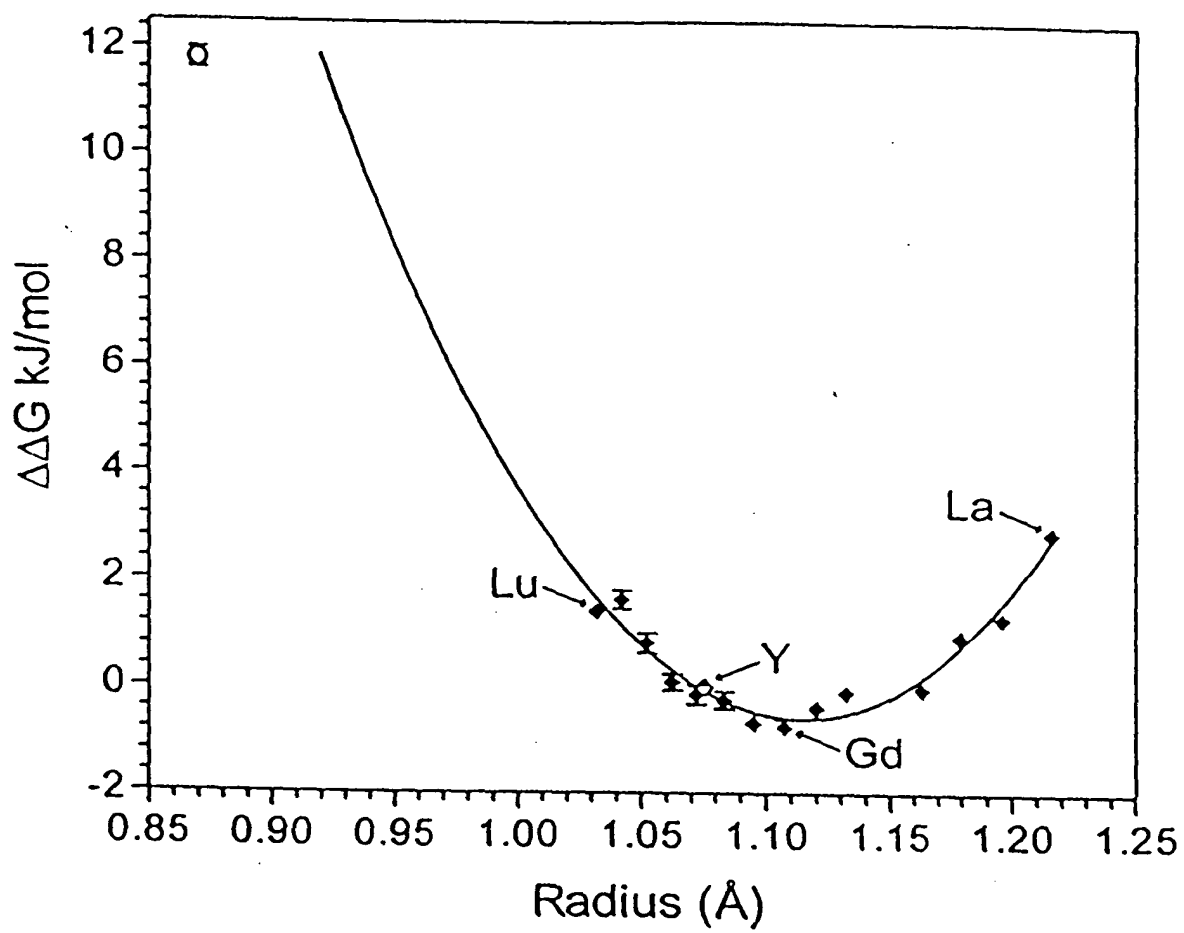


FIG. 15

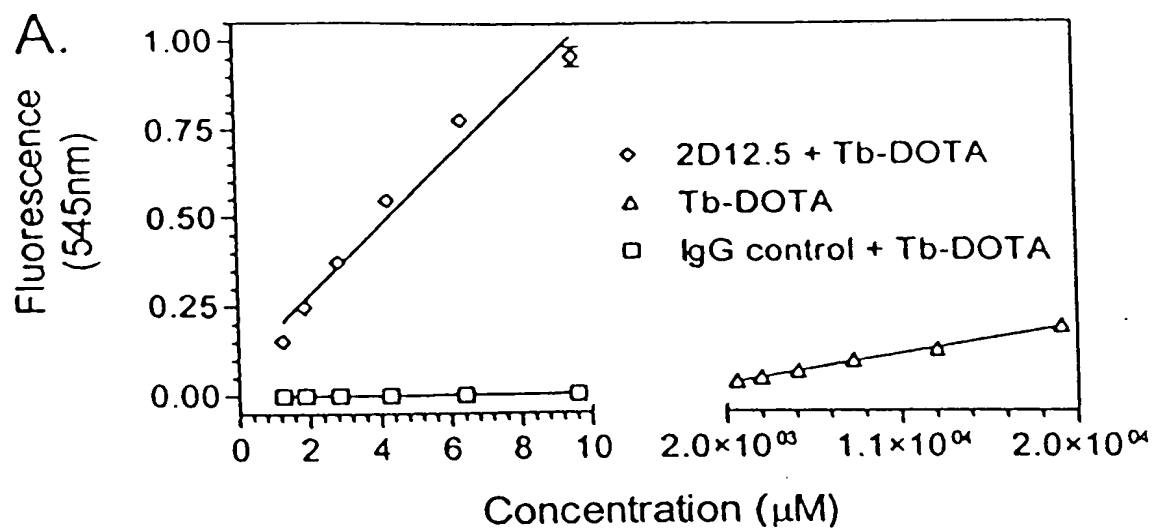


FIG. 16

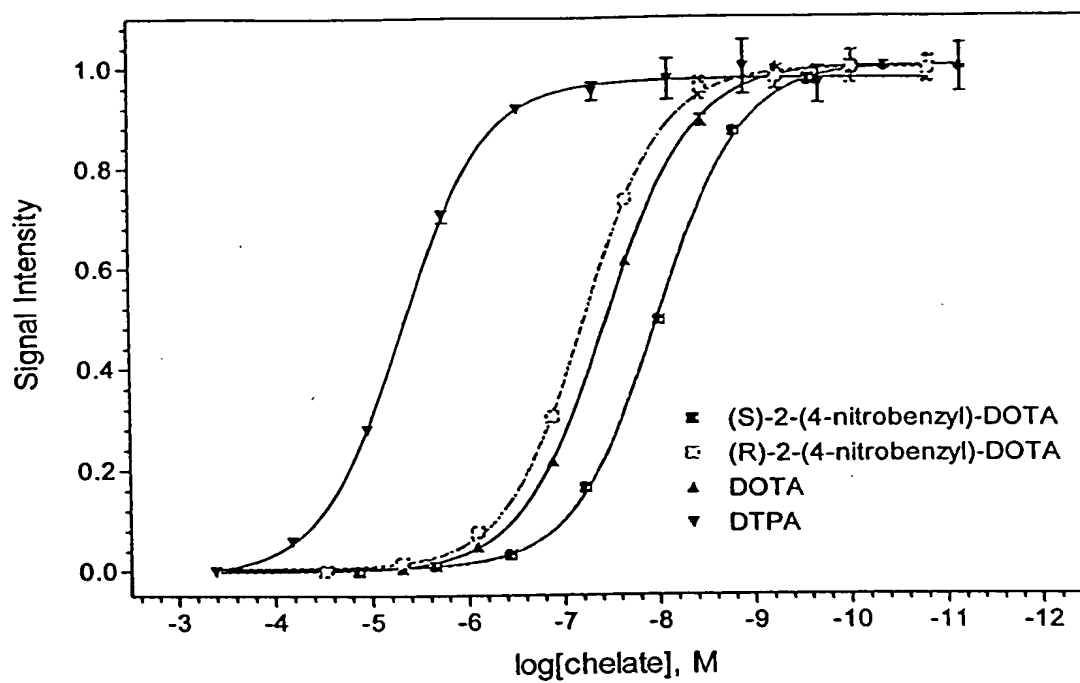


FIG. 17

